REMARKS

Applicant respectfully requests reconsideration of the present application in view of the foregoing amendments and in view of the reasons that follow.

I. Status of the claims

Claims 2, 5, 6, 10, 11, 13, 18, 19, 21, 24, 26-28 and 30 are cancelled. Claims 5, 24 and 26-28 were previously cancelled.

Claims 1, 3, 12, 15, and 20 are amended to clarify the claimed invention and advance prosecution. The amendment to claim 1 incorporates claims 2, 5, 6, 11, 13 (all of which are cancelled), and is further supported by the specification at page 11, last paragraph. Claim 20 is amended to improve clarity. The amendments to the remaining claims are necessitated by changes in their antecedent.

No new matter is added. These amendments are made solely to advance prosecution, without disclaimer of subject matter, and without acquiescence to any rejection.

Applicant respectfully believes that these amendments render the claims allowable and do not require a new search, because they incorporate dependent claims or simplify existing claims.

II. Obviousness rejections

A. The rejections

The Office Action maintains the previous grounds of rejection. In particular, maintaining:

(a) the rejection of claims 1-13, 15, 20-22 and 30 under 35 U.S.C. § 103(a) as allegedly being obvious over Abramson (US Patent Application Publication No. 2003/0077572), in view of Lee (US Patent Application Publication No. 2003/0180710). The Office Action combines Abramson and Lee with additional art to reject the remaining claims:

(b) Kasper (US Patent Application Publication No. 2005/0112706) against claim 14;

(c) Birkemeyer (J. Chromatography A 993: 89) against claims 16 and 17;

(d) MK Hellerstein and RA Neese 1999 *American J. Physiol. Endocr. Metab.* 276: 1146-

1170 against claims 18 and 19;
(e) US Patent Application Publication No. 2004/00811994 A1 to Hellerstein against claim 25;
(f) US Patent No. 5,532,206 to Evans *et al.* against claim 23; and
(g) US 2004/00811994 A1 to Hellerstein with Evans against claim 29.

Applicant believes that the rejection relies, in part, on a misunderstanding of the invention. To advance prosecution, claim 1 is amended to more particularly point out and claim that which is described in the specification.

B. The specification

Prior art methods of analysing metabolic pathways, appropriate for individual pathways, or more defined studies, were not feasible for scaling up for analysis the entire metabolome of a cell. The reasons for these failures differ according to the particular method. For example, when a method of studying the flux of an individual pathway is applied to multiple pathways, the increase in data and the problem of interacting and interfering signals make it impossible, as a practical matter, to both identify and accurately quantify individual metabolites.

In general, applying prior art methods to an entire metabolome (or substantially portion thereof) generates large and complex data sets that could not be deconvoluted in a meaningful way. The solution identified by the inventors is to ensure that the data set is presented in a manner that *is* amenable to deconvolution. The specification teaches a method of obtaining such a data set and can thereby be used for large scale analysis of an entire metabolome. This method combines different technologies known from the prior art, but does so in a manner that is not taught or suggested by the art, and satisfies the unmet need for a technology suitable for metabolome analysis.

One essential feature of the invention is the use of high-saturation isotopic labeling of a first sample. This aids in the identification of unknown metabolites with MS or GC-MS because the number of isotopomers aids in identification of different possible species, e.g. the number of C in a molecule when high saturation ^{13}C is used. Saturation metabolic labeling with U-^{13}C-glucose also allows for the identification of pathways that use glucose and those

that do not, and can be similarly applied to other nutritional sources. In contrast to typical metabolic pathway analysis, in the present invention the saturation metabolic labeling is performed by supplying one or more metabolizable labeling substrates for a time such that the element to be labeled is almost completely replaced throughout the metabolome, i.e., the labeled isotope will be metabolized through all pathways.

The most important advantage of using high-saturation labeling, in the present invention, is that it provides a highly accurate *internal control standard* for use with isotopomer ratios (ITR) to study the impact of changes in conditions on the metabolome. This is done by comparing a first sample, which is isotopically labeled, with a second sample, which is not isotopically labeled. Following chromatography and mass spectrometry, each signal is quantified and provides data that is both highly accurate and internally controlled.

Pairs of signals simultaneously identify species and the change in metabolic pathways in response to a change in conditions via a measure of the ITR between each pair. When doing a paired labeled/unlabeled experiment, you have internal standards for *each* metabolite, and can readily discern changes for all metabolites in response to changes in conditions by changes in isotopomer ration (ITR). Because isotopomers aid in the identification of unknown species, using ITR readily aids in identification the MS species.

The data obtained from the pairs also provide a *total* of the amount of labeled first sample versus unlabelled second sample which provides an independent control for the total amount of first and second samples, and thereby corrects for measurement errors in the amounts of the samples, the amount of isotope label, etc. Thus, the method is robust. Moreover, by providing an additional level of internal control, the data is more accurate and so amenable to deconvolution.

Absolute quantitation of the identified metabolites is done by using a quantatively characterized labeled sample obtained by combining the labeled sample with an unlabeled reference standard or with a well characterized unlabeled sample. The resulting sample is analyzed in the same way as described for any combination of labeled and unlabeled samples.

Because there is parallel and highly accurate and reliable data for an extremely large number of metabolites, and metabolite coresponses, deconvolution can provide an understanding of all (most) pathways and their regulation at a single time.

Finally, the method as claimed can simultaneously resolve metabolites on four axes: (a) chromatography (chemical separation); (b) MS (mass separation of the metabolite and fragments); (c) absolute quantity; and (d) relative quantity (via ITR). It does so, however, without requiring either prior or subsequent steps to identify the species, because the method identifies the metabolite *and* measures the change in metabolic pathways in a single run.

The Examples illustrate how the claimed method is convenient and fast for the quantification of a large number of metabolites from a biological sample. Example 1 demonstrates the feasibility of saturated in vivo labelling of the metabolites in yeast. Example 3 demonstrates that, for a given metabolite contained in a mixture of two samples, the isotopically labelled species can be reliably distinguished from the non-labelled one. Thus, it is possible to determine the relative quantities of the metabolites, in the application referred to as "isotopomer ratio (ITR)". Example 4 shows that the ITR profiling according to the invention provides metabolite data equivalent to the prior art approach where two samples from yeast were separately analysed by GC-MS. Examples 5 and 6 concerns another application of the invention, the generation of mass spectral metabolite tags (MSTs) using GC-TOF-MS for measurement. In the experiment described in Example 6, 180 MSTs were sampled (Table 3) and 78 tags identified, representing 67 yeast metabolites (Table 1). The range of identified compounds comprised amino acids, organic acids, sugars, polyols, purines and pyrimidines, phosphorylated compounds, fatty acids and sterols (Table 1). Furthermore, in Examples 7 and 8, the influence of the various sample preparation methods on the metabolite profile was assessed by applying the method of the invention and co-response analyses. Finally, in Example 9, MALDI-TOF was likewise successfully applied to determine ITRs according to the claimed invention.

Thus, the presently claimed method allows in an unprecedented way to significantly increase the complexity of metabolic data acquisition by enabling the quantification of a large amount of specifically identified metabolites ("at least 50") for a given biological sample. By

comparison, the prior art was unable to specifically identify, and quantify, so many metabolites from a given sample. It follows that the present invention contributes a method by which the limitations of the coverage of metabolome data prevailing in the prior art were overcome.

The presently presented claims have been amended to better explain and reflect the novel contribution of the inventors that is described in the specification. Applicant will now explain how the cited prior art does not render obvious the claims.

C. Abramson

Abramson differs from the method of the invention in that the identity of most compounds analysed remains obscure. Only major components can be identified by Abramson's method as they can only be separated by chromatographic fractionation. Moreover, components of minor concentration can co-elute withmajor components and thereby hamper identification. In particular, while relative differences between samples are determined by combining unlabelled and labelled samples and mass spectrometry, it is clear that the Chemical Reaction Interface/Mass Spectrometry (CRIMS) method of Abramson is quite "unselective" compared to ionization mass spectrometry. In other words, Abramson is uninformative as regards a particular metabolite as in contrast to the present invention. One does not benefit from selective ions or fragment ions (as in the method of the invention), but only utilized unselective combustion products (e.g. CO_2; see Abramson, paragraph [0035]). An ion or fragment ion generated in ionization mass spectrometry is highly indicative ("selective") only for metabolites from which that ion or fragment ion mass or fragmentation ion mass pattern originates. Besides the much higher identification capability of the present invention, the use of additional indicative ions instead of just a retention time range of chromatography, like Abramson, also allows for quantitation. Accordingly, the resolution of the method of the invention is increased by an additional dimension of ion mass compared to using just the low resolution of chromatography as in Abramson. Using the combustion product CO_2 for analysis in the CRIMS device results in low resolution of complex data(i.e. chromatographic separation means only one separating dimension). But because all unseparated components contribute to a common intensity in quantitation in subsequent

biological interpretation, the Abramson method does not allow for substantive interpretation of a particular metabolite. As such, the person of ordinary skill, especially in metabolome analysis, would consider the quantitation of Abramson to be *unselective* compared to the presently claimed method, which adds ionization mass spectrometry. For example, if one using the Abramson method wanted to identify even the *major* proteins that differ between samples, one has to take a fraction from the sample which bears the "isotope enrichment trace" of that compound together with all other coeluting proteins (cf. Figure 1 of Abramson) and perform further analysis to identify the protein. Abramson contains some vague indications in this regard (see e.g. paragraphs [0031] and [0045], claim 16 and Figure 4), however, this can hardly be called enabling of a method of detection, and certainly not one that detects at the same time as quantitation. Thus, although Abramson promises that its method "allows detection and quantification of substances" (see paragraph [0010]), the truth is that the obtained quantitative signal is not attributable to a specific protein. Therefore, the method of Abramson is not selective.

Based on the attached modified Figure 4 of Abramson, we wish illustrate the above-mentioned difference between the claimed method and the method of Abramson. Whereas the prior art method requires the diversion of fractions of the analyzed material and perform subsequent "MS Identification" of fractions of interest, the claimed method directly goes from the chromatographic dimension to the step of MS identification, which at the same time also does the quantification. Thus, according to the method of the invention, it is not necessary to fractionate samples and to perform CRIMS, which would be required following Abramson. Rather, in the present method it suffices to simply quantify the metabolites by analysing the specific isotopic mass differences within the analysed metabolites resulting from the ions, highly selective for the respective metabolite. Thus, the inventors' approach is quite different from Abramson's approach. This explains why the claimed method can be used to quantify *and* identify a large amount of metabolites in a biological sample whereas Abramson's method requires several steps to first quantify in an unselective manner (CRIMS) and second identify separate major component metabolites (MS identification) of the taken fractions (see modified Figure 4). Obviously, Abramson did not believe that a sufficient selectivity can be reached if the whole scale of metabolites is quantified directly by the MS

measurement. Thus, it can be regarded as a real surprise that the data in the present application proves that this can indeed be achieved. In other words, the complexity of the data set covering a broad range of metabolites with one measurement is surprising in view of Abramson. Accordingly, it is clear that there is no incentive or pointer in Abramson to combine this teaching with the teaching of Lee.

D. Lee

As already mentioned previously, Lee has a different goal (flux, time course experiments) than the claimed invention and therefore he does not utilize saturated labelling and does not combine samples, but only determines the isotope label ratio changes within one sample. Lee only analyses very specific metabolites, i.e. those metabolites that are part of specific pathways and their number is very limited. Thus, compared to the claimed invention, the complexity of data is much more reduced. Consequently, a person skilled in the art could not have derived from Lee the expectation that a broad coverage of metabolite data as in the method of the invention could be achievable. It is clear that, without saturated labeling in combination with the determination of the specific isotopic mass differences within the analysed metabolites, Lee cannot analyze and quantify a large amount of metabolites as in the claimed method. This combination which leads to the present invention is not taught or suggested in Lee. Accordingly, there is also no incentive or pointer in Lee to combine its teaching with that of Abramson.

E. Combination of Lee and Abramson

In contrast to Lee's and Abramson's method, the claimed method generates a very complex data set by analysis of specific isotopic mass differences in given metabolites combined with broad metabolite coverage. Based on the methods disclosed by Lee and Abramson, the skilled person could not have achieved the present invention since he/she would have expected that the data generated by the claimed method would be too complex to be handled, too unselective and insensitive and would not aim in reasonable results at the end. Thus, what the prior art (such as Lee and Abramson) *had* to focus on was a less complex setup. The claimed method, by contrast, can be used to analyze and quantify a large amount of metabolites because they are separated by a chromatographic dimension and additionally

by an ion mass dimension of the ions and fragment ions selective for the metabolites. This method is therefore much more selective than Abramson's method based on the unselective combustion products of the metabolites obtained by CRIMS and can, therefore be used to analyse at least 50 metabolites in a biological sample. The results of Lee's method are less complex than that of the method of the invention because Lee's method focuses on a limited number of metabolites (flux analysis).

These limitations were overcome by the present invention which proved as a surprise that a more complex metabolic dataset can be obtained. This solution could neither be derived in an obvious manner from Abramson nor from Lee or a combination thereof.

All of the remaining rejections depend on the combination of Abramson and Lee, which does not render obvious the base claims for reasons explained above, and is not remedied by further combination with the other references. For these reasons, the rejection is believed to be overcome.

CONCLUSION

Applicant believes that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by the credit card payment instructions in EFS-Web being incorrect or absent, resulting in a rejected or incorrect credit card transaction, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicant hereby petitions for such extension under

37 C.F.R. §1.136 and authorizes payment of any such extensions fees to Deposit Account No. 19-0741.

Respectfully submitted,

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